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Toxicity of ingested gamma-1,2,3,4,5,6 benzene hexachloride (lindane) to albino rats

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NOTE TO USERS

TOXICITY OF INGESTED GAMMA-1,2,3,4,5,6 BENZENE HEXACHLORIDE (LINDANE) TO ALBINO RATS

by

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TABLE OF CONTENTS

INTRODUCTION

The advent of synthetic organic insecticides has contributed abundantly to the control of countless insects and closely related arthropods. The effectiveness of these compounds has brought about considerable revision in methods advocated for control of such pests. The results of numerous experimental studies have demonstrated remarkable insecticidal properties of DDT, toxaphene, chlordane, benzene hexachloride and similar organic compounds.

Unfortunately, some of these pesticides were widely used before extensive tests on their toxicity to other forms of life were conducted. The potential hazards to warm-blooded mammals, including man, were recently expressed by the American Medical Association's Council on Foods and Nutrition (1950). The following remarks are taken from a statement made by the Council:

> It is the opinion of the Council on Foods and Nutrition that the information on the following factors should be supplied before pesticides, that may contaminate food or forage crops, are released for general use: (1) Chronic as well as acute toxicity tests. These should be carried out in such a manner as to demonstrate satisfactorily the toxicologic effects of pesticides on warm-blooded animals and man. (2) Accurate methods of isolation and quantitative determination of pesticide residues in biologic material. . . . . Unless this information is supplied before new agricultural poisons are released for general distribution, accidents may occur which will offset the potential benefits of these new materials and cause delay in their adoption. (p. 259)

An editorial in the March, 1950, issue of the Consumer Reports has deplored the fact that the Food and Drug Administration has been reticent about promulgating regulations regarding allowable limits of the quantity of poisonous or deleterious substances used in production of foods, even though the Federal Food, Drug and Cosmetic Act of 1938 was passed by Congress to insure that the total amount of poisons the consumer receives will not be sufficient to jeopardize health. The delay in formulating regulations has been caused by the exigencies of the recent war and the scarcity of information on the toxicity of the new insecticidal agents.

It is a well known fact that, in many instances, substances which are toxic to one living organism are often toxic to other unrelated organisms. It is usually the degree of toxicity that is variable. Thus, the perfect insecticide is one which, in low concentrations, is lethal to the injurious insects and yet, at this concentration, reveals no evidence of toxicity to mammals and other beneficial forms of life.

Included in the category of newer synthetic organic insecticides is benzene hexachloride, more specifically termed 1,2,3,4,5,6 benzene hexachloride or 1,2,3,4,5,6 hexachlorocyclohexane. Thus far, five isomers of this compound have been positively identified. These have been designated as alpha, beta, gamma, delta, and epsilon. Extensive studies have shown that the gamma isomer exhibits the greatest toxicity to insects. Currently, the name "lindane" is

being used to designate the gamma isomer of benzene hexachloride. It has been used frequently in the control of many transient pests as well as the ectoparasitic insects and mites that molest man and domestic animals.

Consequently, it seemed important to investigate the possible toxicity of comparatively low dosages of the gamma isomer of benzene hexachloride to some mammalian species. Most of the literature concerning the toxicity of benzene hexachloride to mammals has been limited to acute toxicity, with less emphasis on chronic toxicity. It is felt that the latter type of toxicological investigation has been neglected, and, as the American Medical Association has pointed out (1950), is imperative.

Penrod and Tauber (1946) made some preliminary observations on the comparative toxicity of benzene hexachloride and of the gamma isomer, alone, using albino rats as test animals. In their experience, at levels between 300 and 600 parts per million of gamma benzene hexachloride in the diet, rats gained weight and did not exhibit any of the classical symptoms of benzene hexachloride poisoning within the duration of the test period. For this reason, the level of 500 parts per million of gamma benzene hexachloride was arbitrarily selected in the following investigations as a basic experimental dietary level for the study of possible chronic toxicity reactions in the albino rat.

This series of experiments herein reported is an attempt to ascertain the effect of prolonged ad libitum ingestion of small quantities of gamma benzene hexachloride to albino rats.

REVIEW OF LITERATURE

Chemical History

Benzene hexachloride[1] was first synthesized by Faraday (1825). He was investigating a new compound, bicarburet of hydrogen, now called benzene. One of the reactions he described involved benzene and chlorine. When this mixture was placed in sunlight, there were formed hydrochloric acid, a dense opaque liquid and a solid crystalline substance. Faraday made no attempt to identify this last product, but future research indicated that the solid substance was, in reality, benzene hexachloride.

Slade (1945) and Guilhon (1946a) have reviewed the early history of the chemical development of benzene hexachloride and its isomers. Currently, there are five known isomers of benzene hexachloride, the latest one isolated being epsilon (Kauer, DuVall and Alquist 1947). According to Slade (1945), the gamma isomer has the greatest insecticidal value. This isomer, as well as the delta form, was discovered by van der Linden (1912).

Guilhon (1946a) pointed out that the term benzene hexachloride is, in reality, a misnomer. The proper chemical nomenclature of this compound is 1,2,3,4,5,6 benzene hexachloride or 1,2,3,4,5,6 hexachlorocyclohexane. However, the continual usage by investigators

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[1] Benzene hexachloride, as used in the present report, denotes a mixture of isomers of benzene hexachloride containing between ten to fifteen per cent of the gamma isomer.

and workers has established the term "benzene hexachloride" as being synonymous with 1,2,3,4,5,6 benzene hexachloride.

Also, as a matter of convenience, the term "lindane" is currently used to designate the gamma isomer of benzene hexachloride, and is officially recognized by the American Association of Economic Entomologists, the Bureau of Entomology and Plant Quarantine, the Food and Drug Administration and various other federal agencies. The editor of Chemical Abstracts accepts the name from the standpoint of chemical nomenclature (Down to Earth 1949). The British investigators have used the trade name "gammexane" to designate the gamma isomer.

Insecticidal Applications

There is disagreement as to who should receive credit for the initial detection of the insecticidal properties of benzene hexachloride. Slade (1945) reported that F. J. D. Thomas, in 1942, had observed that benzene hexachloride was toxic to turnip flea beetles. However, Guilhon (1946a) has disputed this claim. He insisted that, in 1941, Dupre had detected the toxicity of benzene hexachloride to clothes-moth larvae. The matter is still unsettled.

Although the insecticidal value of benzene hexachloride was not ascertained for more than a century after its synthesis, once the testing of this compound was initiated, little time was lost in

experimentation designed to determine the toxicity of benzene hexachloride to numerous species of arthropods. Guilhon (1946a) listed more than fifty different species of economic importance susceptible to benzene hexachloride. Cuff (1946) found that benzene hexachloride was an ovicide as well as an insecticide when used to destroy cattle lice.

The American Veterinary Medical Association's Committee on Parasitology (1949) approved the use of benzene hexachloride for use against ticks, mites and fleas affecting livestock and poultry. However, the committee did not fully approve the use of benzene hexachloride on dairy cattle. They deemed it advisable to withhold judgment until the results of future tests on dairy cattle could be evaluated.

From the standpoint of the entomologist, the most important isomer of benzene hexachloride is gamma (Slade 1945). Gersdorff and McGovran (1945) estimated that the gamma isomer was eight to ten times more toxic to houseflies than DDT. Stewart (1946) and Busvine (1946) found that the gamma isomer was very effective against various arthropods of veterinary importance. Recently, according to Down to Earth (1949), the United States Department of Agriculture has approved the use of lindane (gamma benzene hexachloride) for fly control in dairy barns.

The above few entomological references are included only to emphasize some of the current recommendations of benzene hexachloride

and the gamma isomer as insecticides on warm-blooded animals, and also to note an awareness that their use may involve some danger of toxicity to forms of life other than the arthropodous.

Acute Toxicity

Several investigators have become quite aware of the potential hazard to vertebrates that might be created through the widespread use of this insecticide. The symptoms which accompany the administration of toxic doses to mammals have been described by a number of workers.

Guilhon (1946b) described reactions of sheep, mice and guinea pigs to lethal doses of benzene hexachloride. The first signs of intoxication appeared in the form of muscular spasms which increased in intensity and duration over a period of several hours. The animals became increasingly more irritable. As time passed, the animals had difficulty with locomotion, and ataxia was very evident. After approximately forty hours, they appeared to be in great agony, respirations became shallow, and animals exhibited indications of asphyxia. Following this, they progressed into a comatose state and died.

Furman (1947) described symptoms in mice poisoned with benzene hexachloride. His description of the symptoms was essentially the same as that of Guilhon. Furman noted, in addition, a partial paralysis which was particularly evident in the fore legs. Welch

(1948), experimenting with sheep, reported that these animals were practically blinded by ingestion of high dosage levels of benzene hexachloride. McNamara and Krop (1948a) noted that loss of control of urination and defecation, as well as increased pilomotor activity, frequently accompanied acute poisoning by benzene hexachloride. Lehman (1949b) also described symptoms of acute benzene hexachloride poisoning in mammals. His description summarized information previously reported in the literature.

The quantity of benzene hexachloride that is required to produce symptoms of toxicity has been investigated by a number of workers. Most of these investigations have used albino rats as test animals. Slade (1945) estimated the median lethal dose of benzene hexachloride at 1.25 grams per kilogram of body weight when administered to the rat by a stomach tube over a period of one week. According to Letard and de Saey (1945), the median lethal dose was 3 grams per kilogram of body weight. Guilhon (1946b) estimated the lethal dose for the rat at 5 grams of benzene hexachloride per kilogram of body weight. Taylor and Frodsham (1946) found that growth rates of half-grown rats were not affected if they received oral doses of 500 milligrams of benzene hexachloride per kilogram of body weight. Their experiments were conducted over a period of fifty-seven days.

Many early research workers did not include the percentage composition of the isomers in the benzene hexachloride that they employed in their experiments. The gamma content, for example, may

vary considerably in different benzene hexachloride preparations. Most of the samples seen by this author contained from ten to fifteen per cent of the gamma isomer. The differences in the mixtures may account for some of the inconsistencies in results reported by various workers.

There is considerable difference in the acute toxicity of the isomers of benzene hexachloride. Slade (1945) reported the following quantities as median lethal doses for the rat, measured in grams per kilogram of body weight: alpha, 1.7; gamma, 0.19; and delta, 1.0. Beta was reported non-toxic. Lehman (1948), in comparing acute oral toxicities of insecticides with DDT, listed the following values for isomers of benzene hexachloride, measured in grams per kilogram of body weight: alpha, 0.5; beta, 6.0; gamma, 0.125; and delta, 1.00. Inasmuch as Lehman did not specify the animal he used to obtain his values, it is difficult to compare his figures with those reported by Slade. Both investigators agreed on the positions of relative toxicity of gamma and beta; however, they disagreed on the levels of absolute toxicity. Slade indicated that alpha was more toxic than delta when administered orally, whereas the figures given by Lehman showed a reversal in the positions of these isomers.

The lethal dose of benzene hexachloride when applied dermally has not been determined for the rat, but it is apparent that relatively large quantities are necessary to produce toxic symptoms.

Kirby (1945) painted rats with a five per cent solution of benzene hexachloride over a period of three weeks. Even though these animals were painted with an average of 1.7 grams over the entire period, they remained in a good nutritional state. He observed, however, some retardation in growth. Slade (1945) reported essentially the same results in dermal applications of benzene hexachloride to rats. Chamlin (1946) painted rats with an emulsion containing ten per cent benzene hexachloride and was not able to detect any ill effects from the treatment.

It is difficult to make any generalizations regarding lethal and toxic levels of benzene hexachloride for all mammals. There is a considerable variation in the toxicity of this insecticide to different species of mammals. The work of Guilhon (1946a) illustrated this point. According to him, the oral lethal dose, measured in grams per kilogram of body weight, to guinea pigs was 1.2; dogs, 2.5; sheep, 5.0; and mice, 12.0. Furman (1947), working with mice, found that single oral doses of 1.2 grams per kilogram of body weight were lethal to fourteen out of fifteen mice. Furman's value was quite different from the one reported by Guilhon.

Welch (1948) found that oral administration of 2 grams of benzene hexachloride (ten per cent gamma) per kilogram of body weight produced rather extreme symptoms in sheep, but the animals recovered. He estimated the "maximal safe" dose for sheep at 0.75 gram per kilogram

of body weight. Battle and Turk (1948) found that oral dosages of 100 and 200 milligrams of benzene hexachloride (thirty-three per cent gamma) per kilogram of body weight produced no toxic symptoms in dogs, but at the 500 milligram level, the dogs had diarrhea and exhibited nervousness. Nevertheless, none of his animals died. Lehman (1948) remarked that dogs tended to be more susceptible than rats to injury from insecticides. McNamara and Krop (1948b) found that oral doses of 100 milligrams of gamma benzene hexachloride per kilogram of body weight caused one hundred per cent mortality in rabbits.

Toxic reactions to dermal applications of benzene hexachloride have been studied in rabbits (Purchase 1945) and in mice (Furman 1947). Purchase reported that toxic effects were produced when a suspension of boiled flour and water containing benzene hexachloride was applied at the level of 1.0 gram per kilogram of body weight. When this level was halved, no toxic effects were noted. He also reported that one of his helpers suffered from a transient irritation and reddening of the skin due to handling the suspensions, but this pruritus lasted only six hours.

Furman (1947) demonstrated that the toxicity of sprays of benzene hexachloride to mice varied with the degree of restraint on body cleaning placed on the exposed animals. Mice allowed to lick themselves had a fifty per cent mortality when sprayed with a five per cent solution of benzene hexachloride. If mice were restrained

and not allowed to lick themselves, there was no mortality from the five per cent treatment. A ten per cent spray of benzene hexachloride killed twelve out of thirteen mice, even though they were not permitted to lick themselves.

Not only the amount of restraint, but also the method of dermal application is important in evaluating the toxicity of benzene hexachloride or its pure isomers when applied to the skin. According to Lehman (1948), dermal applications of the gamma isomer were dangerous to mammals at the 4,000 milligram per kilogram of body weight level when applied as a dry powder. When applied in solution, applications were dangerous at the 50 milligram level. Dresden and Krijgsman (1948) expressed the opinion that a median lethal dose of gamma benzene hexachloride for warm-blooded vertebrates is between 300 and 500 milligrams per kilogram of body weight when the isomer is applied dermally.

Intravenously, benzene hexachloride, especially the gamma isomer, seems to possess a rather high degree of toxicity. McNamara and Krop (1948a) reported, in their study on the pharmacology of the isomers of benzene hexachloride, that single intravenous injections of 6 milligrams of the gamma isomer killed twenty-four out of twenty-four rabbits. When the amount was reduced to 4 milligrams per kilogram, no rabbits died. In the same report, they demonstrated that the beta and delta isomers antagonized the action of the gamma isomer. A

mixture of isomers containing 70 parts of alpha, 5 parts of beta, 12 parts of gamma and 7 parts of delta was prepared and injected intravenously into rabbits. Although the amount was equivalent to 94 milligrams per kilogram of body weight, only two of fourteen animals were killed. They further reported that prophylactic injections of the delta isomer protected rabbits from gamma isomer injections.

Chronic Toxicity

There have been a number of studies on the chronic toxicity of benzene hexachloride and its separate isomers to rats. Taylor and Frodsham (1946) fed rats daily doses of 500 milligrams per kilogram of body weight of a mixture of benzene hexachloride (thirteen per cent gamma) over a period of fifty-seven days. They reported the growth rate of these rats to be identical with untreated litter mates, and no toxic symptoms were observed. Slade (1945) reported results of experiments on rats fed 10, 20 or 30 milligrams of gammexane (gamma isomer) per day for five weeks without effects of any kind being produced. Daily feeding of 100 milligrams of a mixture of isomers over a period of two months likewise produced no ill effects.

Lehman (1948) stated that, in rats, the lowest level of the gamma isomer producing gross effects was 400 parts per million in

the food; for alpha, 800 parts per million; for delta, 3,200 parts per million and for beta, 10 parts per million. He emphasized that chronic toxicity sometimes bore no relationship to acute toxicity.

Doisy and Bocklage (1949) found that a diet containing 200 parts per million of gamma benzene hexachloride produced no ill effects, but raising the level to 400 parts per million caused a decrease in the growth rate and caused the rats to exhibit toxic symptoms. When they placed adult rats on a ration containing 800 parts per million of gamma, the animals lost ten per cent of their original body weight over a ten to fourteen day period.

Penrod and Tauber (1946) found that adult rats placed on a ration containing 600 parts of gamma benzene hexachloride per million gained weight. At the level of 900 parts per million of gamma in the food, the males gained weight, but the females lost weight. At levels greater than the 900 parts per million level, both male and female rats lost weight, but the loss in the females was always proportionately greater than that in the males. This suggested a sexual difference in tolerance for the gamma isomer of benzene hexachloride.

Fitzhugh, Nelson and Holland (1949) reported on the chronic toxicity of isomers of benzene hexachloride to albino rats. They performed a series of experiments comparing the chronic toxicity of

alpha, beta, and gamma isomers. Various concentrations were administered orally to the groups of rats under observation. Their results indicated that the beta isomer was more than twice as toxic as the gamma. The alpha isomer was of the same order of toxicity as the gamma isomer. All rats receiving a ration containing 800 parts per million of beta died within ten weeks. Some of the animals receiving the ration containing 1,600 parts per million of gamma survived for more than a year. They detected retardation of growth at the 800 parts per million level for each isomer. At the 100 parts per million level, growth was not affected in any instance. Lehman (1949) also reported on the chronic toxicity of the isomers, but it appeared that he was quoting from the work of Fitzhugh et al (1949).

Furman (1947) studied the chronic toxicity of the gamma isomer to mice. He found no ill effects in eight of ten mice fed 1.5 milligrams of gamma each day for a month. One mouse died on the twenty-first day; the other, on the twenty-second day. The total dose for each surviving animal was approximately 337 milligrams per kilogram of body weight.

It is interesting to note that beta benzene hexachloride appears to be practically non-toxic from the acute toxicity standpoint, yet it possesses the greatest chronic toxicity of all of the isomers of benzene hexachloride. In regard to acute toxicity, the gamma isomer is far more toxic than the alpha. However, when chronic

toxicity is considered, the alpha isomer approaches that of gamma. No plausible explanation for these phenomena has been offered to date.

Pathological Changes

Pathological changes induced by the absorption of benzene hexachloride have been observed by some workers, but reports have been somewhat inconsistent. Slade (1945) found no ill effects in his experiments on the chronic toxicity to rats. Guilhon (1946a) stated that no organic lesions, except some intestinal congestion, were found as a result of benzene hexachloride intoxication. Kirby (1945) observed an occasional gastritis, cloudy swelling in liver and kidney, and, in one instance, degeneration of the suprarenal glands in a male rat. Furman (1947) noted the appearance of gastritis and occasional enteritis in mice fed benzene hexachloride. He frequently found that the intestinal tract was swollen with food and gas. Laug (1948) reported that enlargement of the liver occurred in rats fed on a diet containing 500 parts per million of gamma benzene hexachloride, whether fed for one or four months. He compared the weights of these livers with weights of livers from rats on a ration containing 20 parts per million of gamma. The former averaged 42 grams of liver per kilogram of body weight; the latter averaged 31 grams per kilogram of body weight. Only three castrate

females and three males were included in each group.

Lehman (1948) reported kidney and liver necrosis in animals receiving the gamma isomer of benzene hexachloride. Fitzhugh, Nelson and Holland (1949) observed that, at the 100 parts per million level, alpha, beta, and gamma isomers produced slight, but distinct, histological changes in the liver. The alpha and gamma also affected the kidney. The beta isomer brought about the greatest changes in the liver. At the 10 parts per million level of ingestion, none of the isomers studied produced any damage to the liver except beta, which caused questionable damage.

Lehman (1949) reported essentially the same histological changes as Fitzhugh et al (1949). He also commented on the similarity of pathological changes induced by either benzene hexachloride or DDT. In addition, he noted that alpha and gamma benzene hexachloride caused a moderate degree of hyaline degeneration of the epithelium in the convoluted tubules of the kidney.

Chargaff, Stewart and Magasanik (1948) and Kostoff (1949) showed that the gamma isomer was capable of inducing cytogenic changes and atypical growth in certain plants, and thereby was able to produce tumor-like formations. However, Orr (1948) found that benzene hexachloride had no carcinogenic activity when applied to mammals.

Physiological Effects

Laug (1948) was among the first to ascertain the extent of storage of the gamma isomer in the body of the rat. Using a bio-assay involving Musca domestica, he determined the quantity of this isomer in certain tissues. Fat had the greatest concentration of gamma benzene hexachloride, followed in order by kidney, brain, muscle, and liver. Results on the concentration in the adrenals varied considerably. Appreciable amounts were found in adrenals from castrate female rats, but only traces of the isomer were found in adrenals from male rats. A similar variation was found in the spleen. The amount of gamma found in blood was invariably low, but the isomer tended to be present in measurable quantities even though the animals were on very low dosage levels. On low levels (20 parts per million in food), the gamma isomer was generally not detected in the brain, muscle, or adrenal glands, although small amounts were detected in the liver.

Hixson and Muma (1947) found when poultry were fed on a diet containing small quantities of benzene hexachloride, the muscles of the chickens were so highly tainted as to be inedible. Similar results were obtained by spraying the birds with benzene hexachloride solutions.

Animals are capable, to a limited extent, of excreting benzene hexachloride isomers. Laug (1948), working with rats, found evidence

of the gamma isomer in urine, but little or no gamma was detected in feces. He postulated that the liver was probably quite efficient in metabolizing the gamma isomer. Further, he found that rats, previously on a diet containing 500 parts per million of gamma, placed on a toxicant-free diet for three weeks had no gamma in any of their tissues.

Frawley and Fitzhugh (1949) conducted experiments to determine the rate of excretion of the various isomers. Rats fed diets containing 500 parts per million of gamma or delta eliminated the isomers from fat depots in the body within two weeks after the cessation of ingestion of treated foods. The alpha isomer was still present in fat from rats receiving an equivalent amount in their food. Beta persisted in fat for the longest period of time. Even on a diet containing only 100 parts per million of beta, at the end of two weeks on a toxicant-free diet, less than eighty per cent of the isomer had been eliminated from the fat.

Furman and Hoskins (1948) studied the excretion of benzene hexachloride via the mammary glands in cows sprayed with this insecticide. Within the first week following treatment, the odor of benzene hexachloride was still detected in cream. Essentially the same results were obtained by administering the insecticide *per os*.

It was suggested by Slade (1945) that the toxic action of the gamma isomer of benzene hexachloride might have its origin in an antagonism to inositol. To support this hypothesis, he pointed out

the similarity in the molecular configuration of these two compounds. Evidence has been presented to support Slade's suggestion that gamma benzene hexachloride may counteract the action of inositol. Kirkwood and Phillips (1946) demonstrated that growth in Saccharomyces cerevisiae, which requires exogenous inositol, was inhibited by the gamma isomer. If sufficient inositol was added to the nutrient, growth again occurred. This was also demonstrated by Buston et al (1947) in Nematospora gossypi. Chargaff, Stewart and Magasanik (1948) noted that the arrest in the metaphase of mitosis in Allium cepa, incurred by gammexane treatment, could be inhibited by m-inositol.

However, comparable antagonistic relationships have not been demonstrated in mammals. McNamara and Krop (1948b), working with rabbits, and Doisy and Bocklage (1949), working with rats, were not able to detect any protection against gamma benzene hexachloride intoxication by the utilization of massive doses of inositol.

Dallemagne and Phillippot (1948) suggested that the deleterious effects of poisoning with the gamma isomer might involve cholinesterase. To date, the present author has not found any work to substantiate or deny this hypothesis.

The pharmacological action of benzene hexachloride involves chiefly the central nervous system. Phillippot and Dallemagne (1947) demonstrated that intravenous injections of the gamma isomer caused rabbits to undergo clonic convulsions. Alpha, beta, or delta

isomers did not induce convulsions that are characteristic of the gamma isomer. They contended that the acute toxicity symptoms caused by the gamma isomer were nervous phenomena. They also pointed out that barbiturates were effective in overcoming convulsions caused by gamma benzene hexachloride intoxication.

McNamara and Krop (1948a) confirmed the above results. They pointed out that halogenated hydrocarbons are generally regarded as central nervous system depressants. However, they noted that the outstanding pharmacological action of the gamma isomer was its stimulation of the central nervous system. They further demonstrated the antagonistic action of the beta and delta isomers against the gamma isomer.

The above citations illustrate the imperative necessity of knowing the content of the isomers in technical benzene hexachloride when reporting on toxicity effects.

Comparisons with DDT

Inasmuch as DDT is now such a well known insecticide, and since its action is similar to benzene hexachloride in many respects, it seems pertinent to include certain information regarding the action of DDT on mammals.

Lehman (1948) reported the mean lethal dose of DDT as 250 milligrams per kilogram of body weight when administered orally to mammals. In this same report he estimated that the gamma isomer of

benzene hexachloride was twice as toxic to mammals as DDT. Other isomers were not as toxic as DDT. Welch (1948) considered DDT about as toxic as a mixture of isomers of benzene hexachloride containing ten per cent gamma.

Lehman (1948) listed the lowest levels of certain insecticides that would produce gross effects in rats when administered orally over extended periods of time. With DDT, the figure of 100 parts per million over a period of one hundred and four weeks was given. With gamma benzene hexachloride, the figure of 400 parts per million over a period of fifty-two weeks was listed.

Storage of DDT is chiefly in the fatty tissues of the body. This has been demonstrated by several workers including Woodard, Ofner and Montgomery (1945), Woodard and Ofner (1946), and Laug and Fitzhugh (1946). According to Woodard and Ofner (1946), accumulation of DDT in the fat of rats increased with dietary levels and with length of administration, up to fifty-four days. Ludewig and Chanutin (1946) found that the amount of DDT stored in different tissues reached a maximal level within a few days and remained at a constant level when animals were fed on a 0.1 per cent DDT ration. On the 0.2 per cent level, there was, with time, a progressive increase in amounts of DDT in tissues.

Nelson, Fitzhugh and Laug (1949) noted that DDT was present in the fat at every level of intake down to 1 part per million. There was a progressive rise in the storage of DDT, reaching a maximum at

twenty-three weeks. These same authors noted that fifty per cent of the DDT store in the fat remained after one month on a DDT-free diet. Compared with gamma benzene hexachloride, DDT is apparently excreted at a much slower rate.

The symptoms of DDT poisoning have been thoroughly reviewed by Biskind (1949). These symptoms are quite characteristically the same as those summarized in foregoing paragraphs in relation to benzene hexachloride.

The histopathological changes which accompany absorption of DDT were described by Lillie and Smith (1944), and by Nelson, Draize, Woodard, Fitzhugh, Smith and Calvery (1944). The most noticeable changes were observed in the liver, with some changes in the kidney. Occasionally, histological changes were noted in other organs. Lehman (1949) stated that, in a general way, the pathological picture in benzene hexachloride poisoning resembled the picture in DDT poisoning. Laug and Fitzhugh (1946) found that diets containing 800 to 1,200 parts per million of DDT caused a forty-three per cent increase in the weight of the liver and a ten per cent increase in the weight of the kidneys of rats.

Draize, Nelson and Calvery (1944) reported that the most pronounced change in blood elements accompanying DDT poisoning was a moderate leucocytosis.

Fitzhugh (1948) noted that feeding of DDT to pregnant rats caused a noticeable decrease in survival of young rats subsequently

born to treated females. At the 600 parts per million level of DDT in the food, only 31 per cent of the young were weaned; at the 100 parts per million level, 59.4 per cent were weaned. Female rats on a 10 parts of DDT per million of food successfully weaned 87.5 per cent of their young. Control rats weaned 88 per cent of their young. The effect of gamma benzene hexachloride on weaning of young rats has not been reported in the literature.

EXPERIMENTAL PROCEDURE

General Plan

Albino rats (Mus norvegicus albinus L.) of the Wistar-Ames strain were used in this series of investigations. The rats were selected from a colony which has been maintained by the Department of Zoology and Entomology of the Iowa State College for at least twenty-five years. The rats in this colony are highly inbred.

The animals were kept in a semi-basement laboratory. Rats were housed in rectangular wire-mesh cages of the United States Army Medical School type. Cages, drinking cups and feed pans were cleaned regularly. No attempt was made to sterilize cages and containers, but ordinary sanitation measures were carried out during the course of this investigation.

Rats were permitted to ingest food and tap water ad libitum. The stock ration, prepared by a local feed company, consisted of the following ingredients:

Ground yellow corn	63.75 per cent
Soy bean meal	10.00
Linseed oil meal	8.00
Flour middlings	10.00
Alfalfa meal	2.00
Salt	0.50
Powdered limestone	0.50
Dried skim milk	5.00
Vitamin "A" and "D" concentrate	0.25
	100.00 per cent

The above ration, formulated by Dr. E. R. Becker of the Department of Zoology and Entomology, has been found to be adequate for growth and reproduction without the addition of other supplements.

The experimental diet employed in the investigation herein reported contained 0.5 gram of pure gamma isomer of benzene hexachloride per kilogram of stock ration.[1] This was equivalent to 500 parts per million of gamma in the food. In making up a kilogram of the experimental diet, 0.5 gram of gamma benzene hexachloride was dissolved in approximately 25 milliliters of acetone. This solution was transferred to a beaker containing 100 grams of stock ration. The contents of the beaker were stirred until the liquid was thoroughly distributed through the food particles. After the acetone had completely evaporated, the contents of the beaker were thoroughly mixed with an additional 900 grams of stock ration. It was felt that this procedure yielded a rather homogeneous mixture. Food prepared in this manner will be designated, in this report, as the experimental diet.

In one series of observations a group of animals on the experimental diet received supplemental inositol. The inositol was powdered into fine particles with a small mortar and pestle and mixed, in dry form, with the experimental diet. One gram of inositol

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[1] The gamma isomer of benzene hexachloride was supplied by the Commercial Solvents Corporation, New York 17, New York.

was added to each kilogram of treated food. Thus, the concentration of the inositol was twice as high as the concentration of gamma benzene hexachloride.

Experimental diets were kept in covered battery jars. Because the treated food was consumed by a rather large number of animals, any one preparation of 1,000 grams did not maintain the rats for many days, and any disappearance of the gamma isomer of benzene hexachloride by volatility was likely negligible.

Individual records were kept on all rats used in this study. Although it is a practice of some laboratories to reduce the number of young in large litters to six or seven, this practice was not followed in the present investigation. Young rats were weaned on the twenty-eighth day after birth. At weaning, they were segregated according to sex. Ear marks were used to identify individual animals.

Symptomatology and Mortality

Daily observations were made on all animals included in this study. Any abnormalities in appearance or behavior of individual rats were noted and recorded in the respective records of the rats.

In order to ascertain the effect of prolonged ingestion of small quantities of gamma benzene hexachloride on the mortality rate of rats, thirty-five male and forty-five female rats were kept on the experimental diet for a period of nine months. The animals

were less than ninety days old at the beginning of the experimental dietary period. Thirty male and thirty-two female rats of approximately the same age were used as controls. The fatalities in each group, as well as the causes of death, are listed in Table 1.

Observations on longevity were made on a limited number of rats selected from the experimental and control groups described in the previous paragraph. Fifteen male and eighteen female rats constituted the experimental group; eight male and eight female rats, the control group. Results are summarized in Table 2.

Growth Rates

Inasmuch as the literature contained conflicting reports regarding the effect of gamma benzene hexachloride on the growth rate in rats, it seemed advisable to investigate further this phase of the problem. Three age groups of rats were selected: namely, thirty day, forty-five day and ninety day. In addition to the control and experimental groups in the series of forty-five day old rats, a third set of rats receiving supplemental inositol with the experimental diet was included. Animals came from healthy litters and were paired according to weight and sex. Prior to the initiation of each experiment, the rats to be used were carefully examined and weighed to the nearest gram. Rats exhibiting any abnormalities in respect to growth or general health were discarded.

The thirty day rats were selected from a single litter. Three males and three females were used as experimental animals; two males and two females were used as controls. Observations were made over a period of four weeks. The results are shown in Figure 1.

The forty-five day rats were selected from four litters, all within two days of the same age. The experimental group consisted of six males and four females; the experimental group receiving inositol, five males and four females; the control group, seven males and five females. Observations were made over a period of eight weeks. The results are shown in Figure 2.

The ninety day rats were selected from two litters born on the same day. The experimental group consisted of five males and three females; the control group, four males and three females. Observations were made over a period of four weeks. The results are shown in Figure 3.

Food Consumption and Diet Preference

In conjunction with the study of the growth rate of forty-five day rats described in the previous section, records were kept on the daily food intake of experimental and control rats for a period of four weeks. In addition, the average of the daily weights of each individual animal was determined for each weekly period. The average daily gain for each experimental and control rat was

calculated. Further calculations estimated the approximate amount of gamma benzene hexachloride consumed per kilogram of body weight. The weight gain per gram of experimental or control diet was computed. This procedure was followed in order to compare, in a general way, the level of metabolic activity in the experimental and control groups. The summaries of the results are found in Tables 3 and 4.

An experiment was also designed to determine whether the rat would voluntarily make a choice between the experimental diet and the normal stock ration. Two feed pans of identical dimensions were placed in each cage. One contained experimental food; the other, normal stock food. Daily determinations on the amount of food consumed from each pan were recorded for a period of three weeks. The pans were periodically changed in position to prevent any association patterns from developing during the course of the test period.

Eight weanling rats from two litters were used. Two male and two female rats were from a litter raised by a female on the experimental diet; consequently, these weanlings had been exposed to gamma benzene hexachloride during their uterine and nursing periods of development. Two male and two female weanlings from a normal litter were used as control. Results of this experiment are found in Table 5.

Reproduction and Litter History

Although not a major part of this investigation, data were accumulated on the effect of continued gamma benzene hexachloride ingestion on the reproductive performance of treated rats. The incidence of successful mating was used as a measure of reproductive performance. Observations were made on fifty-three pairings of experimental rats and forty-three pairings of control rats. The results are found in Table 6.

In addition, the mating records of twenty-three experimental males and nineteen control males were examined in order to ascertain the ability of these rats to induce pregnancy. All rats were four to nine months of age. The ability of the experimental and control males to induce pregnancy is given in Table 7.

As a further check on the reproductive ability of male rats maintained on the experimental diet, examinations of spermatozoa from six experimental males were performed at the time of sacrifice. Spermatozoa were obtained by cutting the vas deferens and gently "milking" sperm cells and seminal fluid from the sectioned tube. A drop of semen was transferred to a slide, mixed with physiological saline, and immediately examined. Motility and morphology of the sperm cells were observed. It was assumed that motility and normal structure were indications of viability. The findings are described in the results.

The following data were recorded in evaluating effects of feeding the experimental diet to productive females: age of female, length of experimental diet prior to parturition, period between pairing and parturition, number of young in litter, number of young surviving third day, and number of young weaned. Additional observations were made on the behavior of the mother in caring for her young.

No attempt was made to cage the male and female at the peak of the estrous cycle, and vaginal smears were not prepared prior to pairing. Males were kept with females until signs of pregnancy were evident. At this time, males were removed from the cages. Between the fifteenth and eighteenth day of pregnancy, removal mesh-wire floors of the cages were taken out, and fresh wood shavings were placed in the tray which then served as the cage floor.

Data were taken on three different series of litters. The first series consisted of thirty-seven litters from normal females that had always been maintained on normal food. A second series included forty-four litters from females that had been on the experimental diet prior to parturition, and were continued on the experimental diet after birth of the young. The third series consisted of six litters born of females that had been on the experimental diet, but were placed on normal food at the time of mating. A summary of the data from the first two series appears in Table 8. A complete

history of each litter is located in the appendix. The data from the third series are found in Table 9.

After this study was in progress, it appeared that the percentage of young weaned by experimental females would be relatively small. It was suspected that gamma benzene hexachloride had an inhibiting effect on lactation. To test this hypothesis, four normal litters were observed for a period of two weeks after birth. On the fourteenth day, two of the nursing females were placed on the experimental diet. Weight gains in their litters during the next fourteen days were used as a measure of lactation. The growth rate of the rats in the experimental litter was compared to the growth rate of the rats in the two remaining normal litters. The results are summarized in Table 10.

Hematology

Inasmuch as the literature is devoid of hematological information in chronic gamma benzene hexachloride toxicity, several series of hematological studies were made of groups of animals that had been ingesting the experimental diet.

It has been shown previously that gamma benzene hexachloride induced liver damage in mammals (Lehman 1948). Because the liver plays an important role in stimulating blood cell formation, it was thought that erythrocyte production might be influenced.

Leucocytosis has been reported in DDT toxicity studies (Draize et al 1944). There have been no reports in the literature concerning the effect of gamma benzene hexachloride on the concentration of leucocytes in mammalian blood. Only male rats were used in this study inasmuch as Farris (1942) demonstrated that a leucopenic condition was present at the peak of the estrual cycle in females.

Standard laboratory procedures were followed in both erythrocyte and leucocyte enumerations. Hayem's solution was used as the diluting fluid for erythrocyte counts; two per cent acetic acid was used in leucocyte counts. A Spencer Bright-line hemacytometer was used in all determinations.

Warner (1935) showed that a marked diurnal variation occurred in the concentration of leucocytes in the circulating blood. For this reason, counts were made in the morning between the hours of ten and twelve. In all determinations, tail blood was used. The animal was wrapped in a towel with the tail exposed. Anesthesia was not used. The blood was obtained by cutting off the tip of the tail.

Although a certain error may have occurred as a result of exciting the rats by handling them in the above manner, it was felt that a standard procedure tended to minimize this error.

Erythrocyte counts were made on nineteen animals that had been fed the experimental diet for an average of one year. Twelve rats of corresponding age were used as controls. The results are summarized in Table 11.

A total of sixty-six leucocyte counts was made on animals in different age groups. The period of ingestion of the experimental diet ranged from one month to ten months. The results are summarized in Table 12.

Organ-Body Weight Ratios

Early in the course of this investigation, it was observed, at autopsy, that certain organs of rats that had been on the experimental diet appeared to be somewhat larger than those from control rats. Consequently, a study of the organ weights of rats on the experimental diet was conducted. The organ weights of rats in this group were compared with those from normal rats of similar age, sex and body weight. The organs selected for this study were: liver, kidneys, spleen, and adrenal glands, from both male and female rats. The testes from male rats were also included.

In this investigation, thirty-six experimental and thirty-two control rats were used. Only animals in apparent good health were utilized. Each rat was weighed to the nearest gram prior to sacrifice. The rat was then placed in a wide mouthed jar containing a heavy concentration of chloroform. When respiratory movements ceased,

the animal was removed from the killing jar, and the viscera were exposed by a V-incision. The organs w ere removed in the following order: spleen, left adrenal gland, left kidney, right adrenal gland, right kidney, testes (in males), and liver.

The blood vessels of the spleen were cut close to the hilum and all adhering fat removed. Adrenal glands were dissected from the perirenal fat and all adhering tissue was carefully dissected from the glands. Kidneys were excised intact and, after removal from the body, the blood vessels were clipped next to the hilum, and all adhering fat was removed. In extirpation of the liver, the falciform ligament was first sectioned, and the liver grasped with a pair of forceps at the exit of the hepatic veins into the vena cava. Then, as remaining hepatic mesenteries were separated, the whole liver was gently lifted from the abdominal cavity. Lastly, the portal vein and bile duct were cut near the liver. The testes were pushed through the inguinal canal and removed via the abdominal route. The epididymes were removed and were not included in the weights of the testes.

Absorbent cotton was used to remove excess blood and fluid from liver and kidneys. These organs were placed between two layers of cotton, and gentle pressure applied. Filter paper was used to blot any adhering fluid from other organs.

After all organs were removed, they were weighed individually in the following order: adrenal glands, spleen, testes, kidneys,

and liver. The entire process of dissecting and weighing the organs from a single rat was completed in approximately twelve to fifteen minutes. A chainomatic balance was used in weighing the organs.

Organ-body weight ratios were calculated and expressed as grams of organ per kilogram of body weight. Data on organ weights are summarized in Tables 13 and 14. Complete data on individual rats are given in the appendix.

Histological Study

Histological examinations of the liver, kidney, adrenals and spleen from experimental and control animals were made in this investigation. Samples of tissues were taken from ten experimental animals and five controls. Rats were sacrificed by applying a sharp blow in the region of the occipital foramen. Organs were immediately excised and dipped in physiological saline to remove superficial blood. Ten per cent formalin was used for fixing the tissues. After appropriate treatment, sections were stained with Delafield's hematoxylin and counterstained with eosin. Complete directions for the procedure were taken from "Brief Directions in Histological Technique" by Becker and Roudabush (1939).

Assistance in reading the slides and interpreting the findings was graciously provided by Dr. P. C. Bennett of the Iowa State

Veterinary Diagnostic Laboratory. Descriptions of the findings appear in the results.

Excretion Time

Laug (1946) developed a bio-assay for determining the amount of DDT in animal tissues. The test animal was the house fly. In 1948, Laug also found that house flies could be used similarly in detecting the gamma isomer of benzene hexachloride.

Because of their sensitivity, house flies were used in this study to estimate, qualitatively, the length of time required by the rat to eliminate gamma benzene hexachloride from different tissues after being placed on a toxicant-free diet. The following tissues were used in this study: perirenal fat, kidney, liver, blood, and muscle.

Six female rats were maintained on the experimental diet for one year. One rat was sacrificed on the day of transfer to a toxicant-free diet. The remaining rats were sacrificed successively on the first, second, fourth, ninth, and twelfth day after transfer to normal food.

Approximately a one-half gram sample of each tissue was taken from the rat, macerated, and placed on filter paper six centimeters in diameter. This paper, bearing the tissue to be assayed, was then transferred to the floor of a small cylindrical screen cage approximately ten centimeters high and six centimeters in diameter. Each

cage contained thirty-three flies. The flies were between twenty-four and thirty-six hours old when used for the bio-assay. Water was constantly supplied to test flies.

After twenty-four hours, the number of dead flies was counted. Flies that were unable to walk and apparently moribund were arbitrarily considered dead.

Tissues from normal female rats were tested concurrently with experimental tissues. Also, sugar water alone was supplied to control flies. Inasmuch as the mortality was less than six per cent in flies exposed to normal tissues or sugar water, it was assumed that these were non-toxic to flies. The results of this experiment are found in Table 15.

RESULTS AND DISCUSSION

Symptomatology and Mortality

Generally speaking, rats of the Wistar-Ames strain, when thirty days of age or older, superficially tolerated very well the experimental diet containing 500 parts per million of gamma benzene hexachloride. Experimental males and females appeared to be in good nutritional state even after consuming the experimental diet, in some cases, for more than two years. The fur was well kept; the animals were active, but certainly not hypersensitive. When animals were first placed on the experimental diet, there was a noticeable reduction in food intake for only a few days. However, this decrease in food consumption produced no apparent toxicity symptoms. External symptoms usually described for gamma benzene hexachloride toxicity (convulsions, ataxia, hypersensitivity) were not evident in any of the rats used in this investigation.

The mortality of normal and experimental animals over a period of nine months is given in Table 1. All of these rats were less than ninety days of age at the beginning of the experiment.

It is quite evident, from data in Table 1, that the experimental diet did not cause any appreciable change in the mortality of rats over a nine months test period. The presence of a single death in the experimental female group from a malignant tumor, classified as a squamous cell carcinoma, grade I or II, has

questionable significance. Although hydrocarbons are known to favor the appearance of malignancies, the one instance of tumor in this investigation is not sufficient to incriminate gamma benzene hexachloride. This is consistent with the findings of Orr (1948).

Table 1. Mortality of normal and experimental rats over a period of nine months.

Diet group	No. rats	No. survivals	No. deaths	Cause of death
Exp. male	35	34	1	pneumonia
Cont. male	20	18	2	pneumonia (1) labyrinthitis (1)
Exp. female	45	43	2	pneumonia (1) carcinoma (1)
Cont. female	21	20	1	pneumonia

As in most laboratories, a certain number of rats in our colony contract rat pneumonia. However, in this study, the inclusion of gamma benzene hexachloride in the diet did not seem to contribute toward the incidence of pneumonia; as a matter of fact, the occurence of this lung involvement in the experimental group was lower than in the control group.

Observations on the longevity of a limited number of rats is presented in Table 2. All of these animals were younger than ninety

days when the observations were begun.

Table 2. Longevity of normal and experimental rats.

Diet group	No. rats	Ave. length of exp. diet (days)	Ave. longevity (days)	Range in longevity (days)
Exp. male	15	550	607	406-908
Cont. male	8	-	581	345-722
Exp. female	18	529	558	357-853
Cont. female	8	-	632	531-764

The above results indicated that the present strain of rats was able to tolerate a diet containing 500 parts per million of gamma benzene hexachloride for extended periods. From the data collected it appeared that the male rats, on the average, were better able to survive on the experimental diet than were female rats. This sex difference is the reversal of that in the controls in which the females, on the average, had a longevity of greater duration.

However, it was noted that there was a considerable range in longevity in both experimental and control groups. As pointed out by Griffith and Farris (1942), studies involving observations on older rats are complicated by several interfering factors that are absent in younger (three to four month) rats. Foremost is the prevalence of pneumonia, especially in rats more than a year old. Thus far, we have not succeeded in eliminating this disease from

our colony. It is likely that the variations in longevity reported in this study were due chiefly to the intrusions of certain afflictions, such as pneumonia, rather than to a susceptibility to any substance in the diet.

Labyrinthitis was also evident in several of the older rats in both experimental and control groups. The etiology of labyrinthitis and rat pneumonia is not well understood (Griffith and Farris 1942).

Growth Rates

In Figure 1 growth rates of male and female rats started on the experimental diet at the age of thirty days are compared to the growth rates of litter mates on normal food. The growth rates of forty-five day rats on normal food, experimental food, and experimental diet plus supplemental inositol are shown in Figure 2. The growth rates of ninety day rats on normal and on experimental food are shown in Figure 3.

In all three of the age groups included in this study, a definite pattern in growth rates was evident. Introduction of the experimental ration to rats caused a sharp temporary decline in the body weight. This decrease reached its low point at about the second day following initial presentation of the experimental diet. Following this, weight gains of the experimental animals produced a

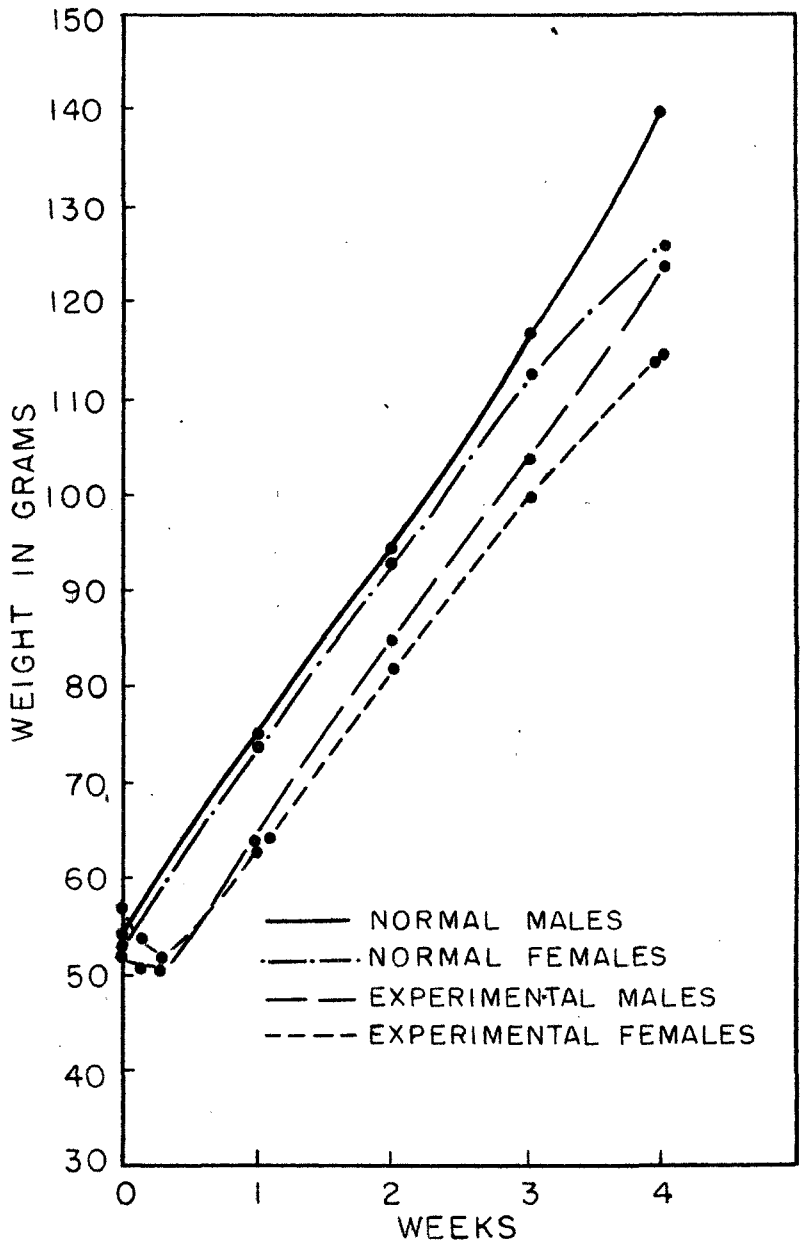

Figure 1. Growth rates in thirty day rats.

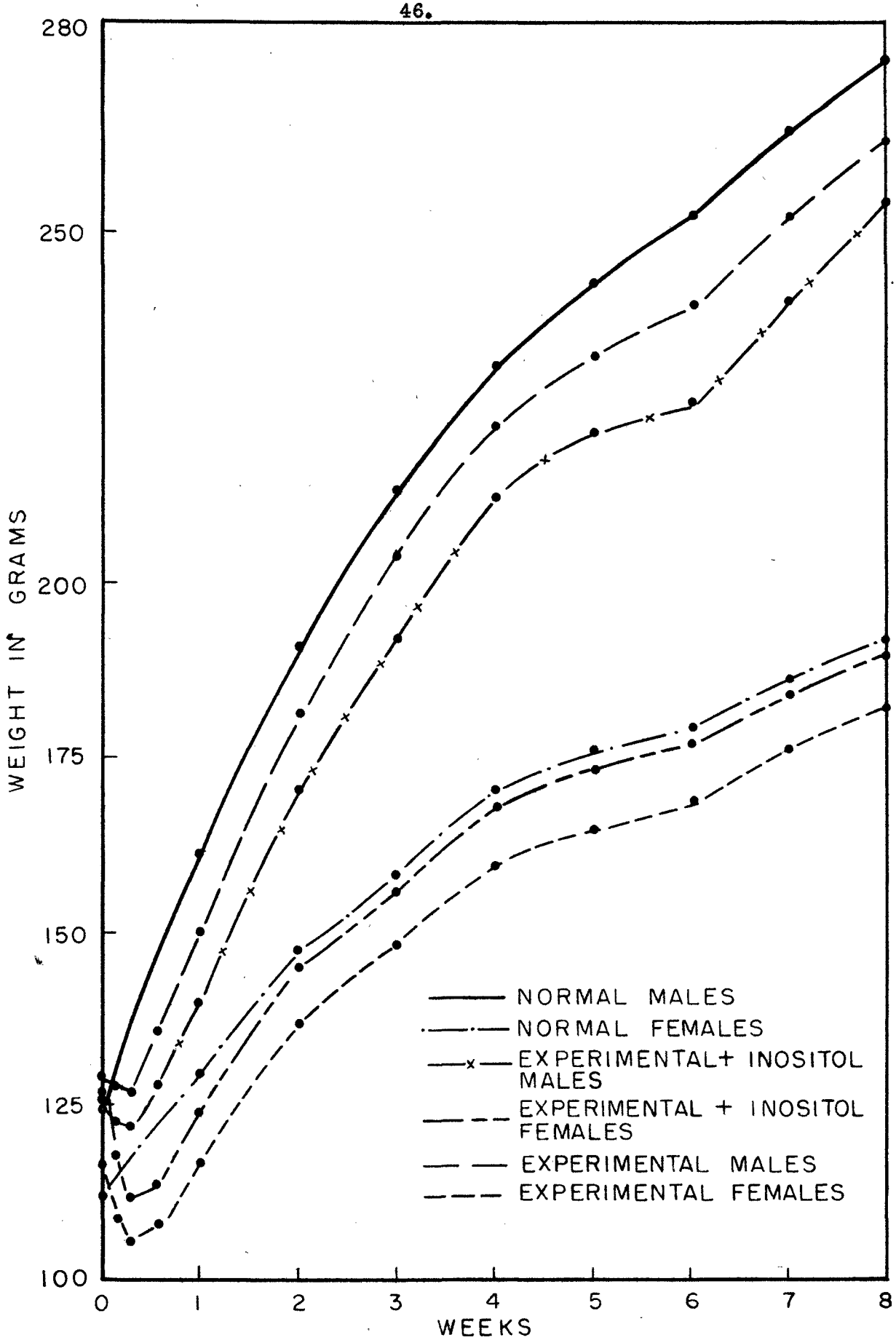

Figure 2. Growth rate in forty-five day rats.

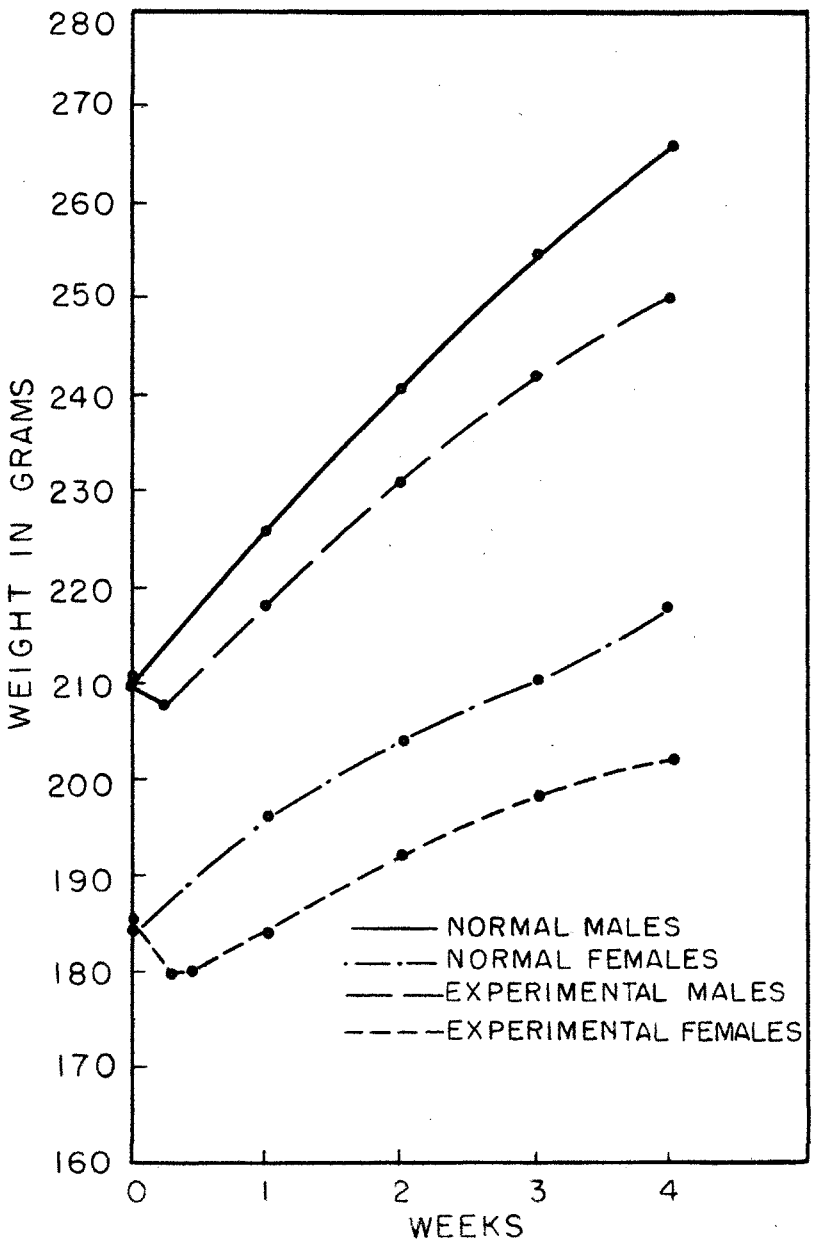

Figure 3. Growth rates in ninety day rats.

normal growth curve. However, within the duration of the test periods, the experimental animals did not equal the body weight of corresponding control animals maintained on the normal stock ration.

On exposure to the experimental diet, the initial weight drop in females was greater than the weight drop in males. Likewise, a longer period of time was required by experimental females to recover their original weight. Males required from three to four days to regain their original weight, whereas females required from four to eight days. Members of the youngest (thirty day) group of rats regained their original weight in less time than did the two older (forty-five and ninety day) groups of rats.

The addition of inositol to the experimental ration did not prevent the initial weight loss. Further, the inositol did not promote a more favorable growth rate in either male or female rats on the experimental diet. This confirmed the report of Doisy and Bocklage (1949). In this study, there was no evidence to support the hypothesis formulated by Slade (1945) who attempted to explain the toxicity of gamma benzene hexachloride on an anti-inositol basis.

In the opinion of this investigator, it is questionable to ascribe the initial weight loss in experimental animals as an indication of a pathological response to gamma benzene hexachloride at the 500 parts per million level. If the first week of the experiment is not included, there is no marked difference between the pattern of growth in normal and experimental animals. As shown

in the following section of results, when rats are first exposed to a diet containing 500 parts per million of gamma benzene hexachloride, they decrease their food intake considerably. Although no difference in the odor of treated and untreated food could be detected by the author, it may be that the olfactory and gustatory senses of the rat are much more sensitive. If this is the case, then the decreased intake is simply a normal physiological response to the presence of a contaminant in the food.

Food Consumption and Diet Preference

The average daily intake of food and the average daily gain in weight of seven normal and six experimental male rats are shown in Table 3. Similar data on food intake and weight gains of seven normal and five experimental female rats are presented in Table 4. In addition, the computed gamma benzene hexachloride ingestion per kilogram of body weight is included for each period.

These data in Tables 3 and 4 revealed a definite decrease in the intake of food by both male and female rats when the animals were first presented the experimental diet. If the first week is not considered, the food intake and the daily gains of experimental rats were similar to the intake and gains in control rats.

When compared to rats on normal food, the relative decrease in food intake during the first week was greater in female than in male

Table 3. Food intake and weight gains in normal and experimental forty-five day male rats.

Diet group	Period	Ave. wt. (gms.)	Ave. da. intake (gms.)	Ave. da. gain (gms.)	Mg. GBHC/ kg. bd. wt.
Exp.	day prev.*	129	17.3	5.58	-
Cont.		126	16.4	4.83	-
Exp.	1st wk.	138	12.5	1.98	46
Cont.		144	16.3	5.20	-
Exp.	2nd wk.	160	16.0	4.12	50
Cont.		174	18.0	3.57	-
Exp.	3rd wk.	187	18.3	3.71	48
Cont.		198	17.3	3.10	-
Exp.	4th wk.	211	19.7	2.95	46
Cont.		220	19.5	3.00	-

* Averages for the day previous to beginning of experiment.

Table 4. Food intake and weight gains in normal and experimental forty-five day female rats

Diet group	Period	Ave. wt. (gms.)	Ave. da. intake (gms.)	Ave. da. gain (gms.)	Mg. GBHC/ kg. bd. wt.
Exp.	day prev.*	115	15.5	2.62	-
Cont.		111	14.5	2.41	-
Exp.	1st wk.	115	8.6	-0.21	37
Cont.		122	14.0	3.04	-
Exp.	2nd wk.	123	13.4	2.57	54
Cont.		139	13.5	1.87	-
Exp.	3rd wk.	140	14.4	2.25	51
Cont.		149	13.9	1.94	-
Exp.	4th wk.	156	14.5	2.28	46
Cont.		163	13.4	1.49	-

* Averages for the day previous to beginning of experiment.

rats, being approximately thirty-eight per cent and twenty-three per cent, respectively.

The decrease in the consumption of treated food is very closely correlated with the initial loss of weight in rats placed on a diet containing 500 parts per million of gamma benzene hexachloride. Thus, with the concentration of gamma benzene hexachloride used in this investigation, it is felt that the initial weight losses are not caused by any pathological reactions.

Further calculations were made to determine the gain in body weight per gram of food ingested. In males, over the entire test period, one gram of food resulted in an increase of 0.20 gram of body weight in the controls; 0.19 gram of body weight in the experimentals. In females, excluding the first week in the experimental group because of weight loss, one gram of food resulted in an increase of 0.15 gram of body weight in the controls; 0.16 gram of body weight in the experimentals.

Apparently the composition of a diet may be a determining factor in the degree of toxicity of gamma benzene hexachloride when it is administered via the food. Doisy and Booklage (1949), using a highly purified ration containing the gamma isomer in melted lard, had a fifty per cent mortality in rats having a body weight between 200 to 300 grams when the animals ate 3.4 to 4.1 milligrams of gamma benzene hexachloride per day. In the present investigation, using

a diet of natural foodstuffs containing the gamma isomer in dry form, the male rats, with body weights between 125 and 225 grams, averaged a daily consumption of 8.3 milligrams gamma benzene hexachloride, and no deaths occurred during the test period. Likewise, female rats on the experimental diet averaged a daily intake of 6.3 milligrams of gamma benzene hexachloride with no mortality. It is questionable that a difference in strains of rats could account for the highly different results in the two studies.

A measure of the ability of rats to distinguish between treated and untreated food is indicated in Table 5. Weanlings from a litter nursed by a female on the experimental diet are designated as experimental rats; weanlings from a normal litter are designated as control rats.

Table 5. Dietary preference of weanling rats.

Group	No. rats	1st week		2nd week		3rd week	
		Daily intake (gms.)	% exp. diet	Daily intake (gms.)	% exp. diet	Daily intake (gms.)	% exp. diet
Exp. male	2	15.8	15.7*	19.2	10.5	18.6	6.1
Cont. male	2	16.4	4.0	18.4	2.0	18.4	2.0
Exp. female	2	11.4	30.0	11.6	28.0	11.1	24.0
Cont. female	2	11.5	16.0	11.7	10.4	13.1	7.0

* Per cent of treated food ingested in relation to total daily intake.

In all instances, rats showed a preference for the untreated food. The control weanling males were particularly sensitive to the treated food. The experimental females selected a greater percentage of treated food than any other group. With time, all animals selected less of the experimental diet.

The data of Table 5 illustrate that rats can apparently detect small quantities of gamma benzene hexachloride in food. Accidental poisoning of rats by ingestion of large quantities of gamma benzene hexachloride seems very unlikely.

Reproduction and Litter History

The mating success of fifty-three pairings of experimental males and females, and of forty-three pairings of control males and females, are summarized in Table 6. The experimental dietary period prior to mating ranged from nine to three hundred days.

Table 6. Mating success of normal and experimental rats.

Diet group	No. of pairings	No. of pregnancies	Successful mating %
Exp.	53	44	83
Cont.	43	37	86

In this study, there was very little difference between the mating success of normal and experimental rats. In both instances, the percentage of successful matings was relatively high.

Examination of the mating record of the normal and experimental males used in the course of this study was made. The results of this examination are shown in Table 7.

Table 7. Reproductive ability of normal and experimental male rats.

Diet group	No. males	No. of males proved potent	Potency percentage
Exp.	23	21	91
Cont.	19	17	89

The potency of male rats was not reduced when they were maintained on a diet containing 500 parts per million of gamma benzene hexachloride.

In the course of this study, spermatozoa from six males that had been on the experimental diet for six months prior to sacrifice were examined. In all instances, sperm motility was evident. Morphologically the sperm cells appeared to be normal. These findings were consistent with the results given in Table 7.

The high incidence of successful mating was probably due to a careful selection of breeding stock. Vigorous animals were always selected for breeding purposes. Thus, a random selection of rats used for breeding was not made in this investigation.

A comprehensive study was made of maternal activities of female rats subjected to a diet containing 500 parts of gamma benzene hexachloride. A summary of the data is given in Table 8.

Histories of individual litters are found in the appendix.

Table 8. Histories of litters from normal and experimental females.

Diet group	Age (mos.)	Ave. exp. diet (mos.)	No. litters	Pair. to partur. (days)	Ave. no. in litter	Ave. surv. 3 days	Ave. no weaned
Cont.	3-6	-	15	23.5*	8.2	7.0	5.9
Exp.	3-6	3**	22	24.3	6.6	1.6	1.1
Cont.	6-9	-	18	25.9	8.4	7.6	7.1
Exp.	6-9	5½	17	25.0	6.3	0.4	0.2
Cont.	9-12	-	4	28.0	7.9	5.2	3.7
Exp.	9-12	9	5	27.0	5.6	1.4	0.4

* Average number of days between pairing and parturition.
** Average length of experimental diet prior to parturition.

The data from Table 8 were treated statistically by using the t-test of the null hypothesis as outlined in Statistical Methods by Snedecor (1944).

Statistically, there was no significant difference in the duration of the pairing to parturition period in females in the same age group. Differences in the size of normal and experimental litters were significant; in the three age groups the t-values were located between the 1 % and 5 % level. In respect to survival at the end of three days and survival at weaning, the differences between normal and experimental litters within the same age group were highly significant.

Measured by the ability to bring young rats to weaning age, there was no evidence that female rats developed a tolerance for the gamma benzene hexachloride. In all cases mortality by weaning time was exceedingly high. Experimental females that were on the experimental diet for the shortest period succeeded in maintaining a higher percentage of the young until weaning.

The decrease in litter size from experimental females was somewhat surprising in view of the fact that the mating success of experimental rats compared favorably with the mating success of control rats. There was one possible source of error that may have been responsible for this apparent difference. Rats have a tendency to cast their litters during the night. In the experimental group, the practices of infanticide and cannibalism were not uncommon. Thus, it is quite conceivable that some of the young may have been killed and eaten before the observer had an opportunity to examine the litter on the day following birth.

The failure of the experimental females in rearing their young is striking. The addition of gamma benzene hexachloride to the diet apparently has a drastic influence on the maternal performance of the female rat. Fitzhugh (1948) has shown that DDT exerts a similar influence on the ability of the female to care for her young.

Although most litters are born at night, this investigator was fortunate enough to observe a number of experimental females in the process of giving birth to their young. In all instances observed,

the young were invariably born alive. Furthermore, this observer was not able to detect any symptoms in the new-born rats that indicated a toxic condition. They appeared to be perfectly normal.

It was suspected that the new-born rats might be poisoned by ingestion of the mother's milk which presumably contained gamma benzene hexachloride. However, the condition of the nipples in unsuccessful experimental mothers indicated that the young had never suckled. In addition, the absence of milk in the stomachs of the young which had died within the first twenty-four hours also indicated a failure in nursing.

An excellent monograph on maternal behavior in the albino laboratory rat has been written by Wiesner and Sheard (1933). In the present investigation deviations from normal maternal behavior pattern were noticeable in females on the experimental diet. Parturition seemed to be normal, and the females consumed the placenta and fetal membranes following birth. However, females on the experimental diet often made no attempt to retrieve the young and to assemble them in a nest. Characteristically, the young were scattered about the rearing cage and neglected. Wiesner and Sheard (1933) described normal behavior patterns in lactating female rats. They noted that the female needed to sit still in order for the young to suckle successfully. A "nursing posture" was maintained by the female to allow the young to nurse without being squashed. This characteristic was absent in many of the unsuccessful

experimental females. In numerous instances, the young appeared to have suffocated, for they seemed to form an integral part of the bed for the reclining female.

Sica and Cerecedo (1948), in their study on the nutritional requirements of the rat for reproduction and lactation, expressed the opinion that young rats which failed to survive the third day of lactation should not be classified as lactation failures. They believed that such deaths could be traced to disorders in the process of reproduction. In their opinion, the dietary requirements during pregnancy are as great as those during lactation. They further doubted the existence of a specific dietary factor required for lactation only. If the data of Table 8 are examined in the light of Sica and Cerecedo's opinion, it would be necessary to conclude that the low survival of young born to experimental females was due to basic difficulty in the reproductive process.

Although the evidence is highly circumstantial, the behavior of the females receiving the experimental diet somewhat suggests a disturbance in manganese metabolism. Orent and McCollum (1931) (1932) fed rats on diets practically free of manganese and noted that the animals grew to maturity; the females had normal estrual cycles, and when mated, produced litters of normal size. However, these females were unable to suckle their young. Perla (1939), and Perla and Sandberg (1939) reported an interdependence of manganese and thiamin. They noted that female rats fed diets containing high

supplemental amounts of thiamin exhibited cannibalistic tendencies, loss of maternal instincts, interference with lactation and a progressive loss of fertility. These symptoms were offset by administering small amounts of manganese to the deficient female.

For the most part, the above symptoms of manganese deficiency were noted in the experimental females studied in this investigation. To the writer's knowledge, the internal mechanism of manganese metabolism has not been elucidated. It is possible that the continued administration of gamma benzene hexachloride may cause a disturbance in manganese metabolism. However, manganese deficiencies are said to cause sterility in male rats, and also cause a retardation in growth. These symptoms were lacking in rats observed in this investigation. The possibility of an interrelationship between gamma benzene hexachloride and manganese metabolism requires a great deal more investigation.

An experiment was designed to test the permanency of the abnormal maternal behavior in experimental females. Experimental females with poor litter histories were placed on normal food at the time of a subsequent pairing with a normal male. The performance of the six female rats used in this experiment is shown in Table 9.

The results showed that the detrimental effect of gamma benzene hexachloride on the maternal performance of female rats was

not permanent. When experimental females with poor litter histories were placed on a normal ration at the time of the subsequent pairing, they were able to nurse seventy-three per cent of their young to weaning.

Table 9. Maternal performance of individual rats before and after discontinuation of experimental diet.

Rat no.	On experimental diet		On control diet	
	Litter size	No. weaned	Litter size	No. weaned
E-22	8	0	8	8
E-40	9	0	7	0
E-41	4	0	11	10
E-42	7	0	10	7
E-43	11	0	10	9
E-44	9	0	10	7
Average	8.0	0.0	9.3	6.8

The immediate effect of a diet containing 500 parts per million of gamma benzene hexachloride on lactation was determined on two lactating females. When their litters were fourteen days old, the females were placed on the experimental diet. The growth rate of the nursling rats was used as a criterion of lactating ability. Two lactating normal females with litters were used as control. The results are given in Table 10.

From these data, it appeared that the gamma benzene hexachloride did not have any immediate effect on the lactating ability of female rats. The weight gains on rats from experimental litters compared favorably with the weight gains of rats from control litters.

Table 10. Growth of nursling rats when mother is placed on experimental diet.

Group	No. of litters	No. nurslings	Average weight of nurslings (grams)		
			14 da.	21 da.	28 da.
Exp.	2	16	20.1*	29.3	46.5
Cont.	2	16	19.2	30.5	47.2

* Nursing female placed on experimental diet on this day.

It must be kept in mind that this experiment tested only the immediate effect of gamma benzene hexachloride on lactation. It is entirely possible that the ingestion of gamma benzene hexachloride over long periods may affect lactation.

Hematology

The concentration of erythrocytes in the blood of normal and experimental rats is given in Table 11.

No significant change in the erythrocyte concentration in rats was induced by the addition of 500 parts per million of gamma benzene hexachloride to the diet.

Table 11. Erythrocyte counts in normal and experimental rats.

Group	No. rats	Ave. length of diet (mos.)	Ave. no. RBC per cu. mm.	Range
Exp.	19	12	9.04*	7.70-12.39**
Cont.	12	-	8.95	7.17-12.87

* Number of cells expressed in millions.
** Range in number of cells expressed in millions.

Data relative to the number of leucocytes per cubic millimeter of tail blood in normal and experimental male rats are assembled in Table 12.

Table 12. Leucocyte counts in normal and experimental male rats.

Group	No. rats	Age (mos.)	Length of exp. diet	Ave. no. cells per cu. mm.	Range
Exp.	8	3	1*	8.34**	4.96-10.64***
Cont.	6	3	-	9.52	5.08-12.60
Exp.	7	4½	3	11.98	10.97-14.88
Cont.	3	4½	-	10.97	8.28-12.48
Exp.	11	6	4	9.93	7.28-15.28
Cont.	14	6	-	10.80	7.40-17.92
Exp.	10	12	10	10.98	7.80-14.52
Cont.	7	12	-	10.03	6.65-14.08

* Length of diet expressed in months.
** Number of cells expressed in thousands.
*** Range in number of cells expressed in thousands.

Although it appeared that there was some difference in the concentration of leucocytes in several groups, the variation within each group was so large that statistical tests uncovered no significant difference between the control and experimental groups.

Griffith and Farris (1942) have stated that the adult rat averages 9,000 leucocytes per cubic millimeter of blood with a normal range between 6,000 and 18,000. All rats of the present investigation in age levels above three months had normal leucocyte counts. This investigator did not detect leucocytosis in the rats (males, only) maintained on the diet containing 500 parts per million of gamma benzene hexachloride. Leucocytosis has been reported in DDT toxicity studies (Draize et al 1944).

Several other tests were performed on a few rats, but were abandoned when it became obvious that no significant differences were being encountered. Hemoglobin levels were within normal limits. The icteric indices of experimental rats were normal. The plasma protein level in experimental rats was similar to the level found in control animals.

Organ-Body Weight Ratios

As previously noted in the review of literature, Laug (1948) reported that livers of animals ingesting a diet containing 500 parts per million of gamma, whether for one month or four, were found to be definitely enlarged when compared with livers of animals

ingesting a diet containing 20 parts per million of gamma. He reported an enlargement of approximately twenty-five per cent. Only three males and three castrate females were used by Laug. In the present investigation, organ-body weight ratios were determined for thirty-six experimental and thirty-two control rats. The data concerning the organ-body weight ratios on male rats are given in Table 13; data on female rats are given in Table 14. Complete records of individual rats are found in the appendix.

Table 13. Organ-body weight ratios of male rats.

Group	Age (mos.)	Exp. diet (mos.)	No. rats	Ave. wt. (gms.)	Ave. gms. organ/kgm. body weight				
					Liver	Kidneys	Spleen	Adrenals	Testes
Exp.	$3\frac{1}{2}$	2*	3	235**	50.9	11.6	2.10	0.12	12.1
Cont.	$3\frac{1}{2}$	-	3	234	43.7	8.8	2.17	0.11	12.2
Exp.	7	6	11	338	40.6	10.3	1.95	0.10	9.6
Cont.	7	-	12	338	35.1	8.3	1.72	0.10	10.8

* Duration of experimental diet prior to sacrifice.
** Average weight at time of sacrifice.

The increase of the relative weight of the liver of experimental rats over control rats of the same age was highly significant; t-values were 1 % or better. The increase of the relative weight of the kidney of experimental rats over control rats of the same age was highly significant in all age groups of male rats and in the seven month female rats. In the eighteen month female rats, the difference was less significant; the value of t was between the 2 % and 5 % level. In three month female rats, there was a trend toward

an increase in the relative weight of the kidneys in the experimental group, but the value of t was greater than 5 %.

Table 14. Organ-body weight ratios of female rats.

Group	Age (mos.)	Exp. diet (mos.)	No. rats	Ave. wt. (gms.)	Ave. gms. organ/kgm. body weight			
					Liver	Kidneys	Spleen	Adrenals
Exp.	3	2*	6	195**	50.3	9.3	2.67	0.25
Cont.	3	-	6	188	41.3	8.9	2.68	0.27
Exp.	7	6	4	197	43.0	8.9	2.02	0.22
Exp.-N	7***	3	4	218	39.3	8.7	2.40	0.21
Cont.	7	-	7	211	35.0	8.1	1.89	0.18
Exp.	18	17	8	213	44.4	10.9	1.98	0.19
Cont.	18	-	4	238	34.7	9.4	2.30	0.17

* Duration of experimental diet prior to sacrifice.
** Average weight at time of sacrifice.
*** Animals were on normal food 1 month, then experimental diet 3 months, then normal food remaining 3 months.

The liver and kidneys from female rats that were on the experimental diet for three months, and then placed on normal food for three months prior to sacrifice, were relatively larger than corresponding organs in control rats, but were relatively smaller than corresponding organs from experimental rats of the same age.

The relative increase in the weight of the kidneys from experimental male rats was greater than the relative increase in experimental female rats.

The spleen weight from seven month females that were on the experimental diet and then shifted to the control diet three months prior to autopsy was significantly greater than either the spleen

weight from control or experimental females from the same age group. No explanation is offered for this difference; however, the number of rats concerned (four) was rather small. With the exception of the spleen weight in the above group, no significant differences were found in other spleen, adrenal or testis weights.

In this study, a special effort was made to use rats of similar sex, age and weight in order to alleviate errors that might arise in random sampling. Cameron (1925) has pointed out that generally the relative organ weights of liver, kidneys and spleen tend to decrease with an increase in body weight. Walter and Addis (1939) also noted this decrease in relative weights of liver, and kidney and, in addition, testis. Even in experimental rats, these tendencies were generally evident in the present investigation.

In the current study, variations in the weights of a particular organ were often large, even in litter mates. However, this situation occurs quite frequently, as pointed out by Webster, Liljegren and Zimmer (1947). For this reason, it seems essential that statistical methods be used to analyse pertinent data.

It is of interest to note that, of the organs included in this study, the two which showed the greatest increase in relative weight were the liver and kidneys. It is generally known that these organs are active in detoxification processes. The hypertrophy of these organs would seem to be a physiological response to the

presence of gamma benzene hexachloride in the circulating blood. Laug (1948) suggested that the liver is particularly efficient in metabolizing the gamma isomer. The current study indicates that the kidney might also be active in metabolizing this isomer. The results on growth rates and longevity of experimental rats indicated that males tended to tolerate gamma benzene hexachloride better than females. The relative increase in the liver weights were approximately the same for both experimental males and females, but the relative increase in the kidney weights was much higher in the males (24 to 32 %) than in the females (4 to 16 %). Perhaps the apparently better tolerance of gamma benzene hexachloride by male rats is traceable to a greater hypertrophy of the kidney.

Histological Study

The findings from histological examinations of the liver and kidneys from experimental rats maintained on a diet containing 500 parts per million of gamma benzene hexachloride were essentially the same as those reported by Lehman (1948) and Fitzhugh, Nelson and Holland (1949). The liver was most noticeably affected. The damage varied from occasional cloudy swelling to fatty degeneration and central necrosis. Evidence of chronic toxicity was indicated by degenerative changes in the liver (albuminous and fatty degeneration). However, in only one out of ten experimental animals was the damage extensive enough to be considered irreparable. In this one

case, fatty degeneration was found around the periphery of the lobules; central necrosis, with hydropic degeneration and karyolysis, was found in the middle of the lobules. This male rat had been on the experimental diet for seven months. Two of the ten animals were on the experimental diet for a year; only initial stages of fatty degeneration were found in their livers.

Generally, the kidneys appeared to be quite normal. Evidence of toxicity in the kidneys of two animals was indicated by dilatation of the tubules, and accumulation of hyaline material.

The adrenals and spleen appeared normal in all sections examined.

Excretion Time

The time required for the depletion of tissue gamma benzene hexachloride was estimated by bioassay. When the toxicant in the tissues from experimental females, currently on normal food, killed less than six per cent of the flies, it was assumed that the amount of toxicant in the tissue was negligible. The results are shown in Table 15.

Although the results were only qualitative, they indicated that female rats were able to eliminate gamma benzene hexachloride from their tissues very rapidly. These results agreed essentially with the findings of Laug (1946), who detected no gamma benzene hexachloride in rat tissues after three weeks on a toxicant-free

diet, and Frawley and Fitzhugh (1949) who found that gamma was eliminated from the fat within two weeks after the cessation of ingestion of a diet containing 500 parts per million of gamma benzene hexachloride. The elimination of DDT from tissues is slow compared to the disappearance of gamma benzene hexachloride. Nelson, Fitzhugh and Laug (1949) found that fifty per cent of the DDT still remained in the fat after one month on a DDT-free diet.

Table 15. Bioassay of residual gamma benzene hexachloride in tissues of rats returned to a normal diet.

Rat	Normal food (days)	% Mortality in flies exposed to tissue				
		Fat	Kidney	Liver	Blood	Muscle
N-0	0*	94	66	24	6	9
N-1	1	91	51	21	3	3
N-2	2	51	15	12	0	0
N-4	4	78	21	0	3	-**
N-9	9	15	0	3	-	-
N-12	12	15	6	0	-	-
Cont.	-***	6	3	3	0	0

* Number of days on normal food following ingestion of experimental diet for one year.
** No assay conducted.
*** Control rats had not been subjected to experimental diet.

Using the mortality of house flies as a measure of the presence of appreciable amounts of gamma benzene hexachloride, it appeared that this toxicant was eliminated from the muscle within two days. The amount of gamma benzene hexachloride was too low to be measured after the first day; likewise, the amount in the liver was too low to

be measured after the second day. Detectable amounts were found in the kidney and fat even on the twelfth day; however, the per cent mortality of the flies indicated that the amount was very low.

Because of the nature of the test, the author does not wish to imply that all gamma benzene hexachloride is absent when the mortality of flies is low or negative. It is felt that this bio-assay method is not that accurate. For example, if the toxicant is being eliminated from fat, kidney and liver, and if these organs continue to show measurable quantities, then it is only logical to assume that the toxicant is also in the blood. However, results in Table 15 are not altogether consistent with this line of reasoning.

SUMMARY AND CONCLUSIONS

1. An investigation of the chronic toxicity of a diet containing 500 parts per million of gamma benzene hexachloride was made on albino rats of the Wistar-Ames strain.

2. Weanling or older rats tolerated very well the experimental diet for a nine month test period. However, studies on longevity of rats fed on a diet containing 500 parts per million of gamma benzene hexachloride showed that male rats survived longer than female rats. In contrast, in animals maintained on stock ration, females outlived males.

3. The introduction of the experimental diet caused an initial loss in body weight of rats. The loss of weight in female rats was relatively greater than the loss in male rats of the same age. After the first week, the growth rate of experimental animals was similar to the growth rate in control animals. However, within the duration of the test periods, experimental animals did not equal the body weight of corresponding control animals.

4. The addition of inositol to the experimental diet did not alter the growth rate of experimental animals. Evidence obtained in this study did not support the hypothesis that, in mammals, gamma benzene hexachloride counteracts the action of inositol.

5. When rats were first exposed to the experimental diet, there was a decided decrease in the food intake. This decrease was closely correlated with the initial weight loss following introduction of experimental food. After the first week, the food intake of experimental rats was similar to the food intake of controls.

6. In forty-five day rats, equivalent amounts of food produced similar increases in body weights in both experimental and control rats. Male rats averaged a daily consumption of 8.3 milligrams of gamma benzene hexachloride; female rats averaged 6.3 milligrams of gamma benzene hexachloride. Other investigators, using purified diets, have reported fifty per cent mortalities when rats received approximately one-half of this amount of gamma daily. This indicates that the composition of a diet may be a contributing factor in the extent of toxicity symptoms developing from ingested gamma benzene hexachloride.

7. Weanling rats demonstrated an ability to differentiate between treated and untreated food. When given a choice between normal and experimental food, the animals chose greater quantities of the untreated food. Male rats were apparently able to detect the presence of the gamma isomer more readily than female rats. With time, weanling rats given a choice between treated and untreated food showed a progressive decrease in the percentage of treated food ingested.

8. Using the incidence of successful mating as a measure, the reproductive ability of experimental animals was not altered appreciably by prolonged ingestion of a diet containing 500 parts per million of gamma benzene hexachloride. However, females maintained on experimental food for long periods previous to parturition had significantly fewer rats per litter than corresponding control females.

9. Experimental females were highly unsuccessful in rearing their litters. For example, three to six month old females when fed experimental food for an average of three months prior to parturition were able to rear approximately sixteen per cent of their young to weaning; corresponding control females succeeded in rearing approximately seventy-three per cent of their young to weaning. Further, experimental females showed no increased tolerance when placed on experimental food for periods longer than three months. It was also noted that the inclusion of gamma benzene hexachloride in the diet caused a considerable change in the maternal behavior in female rats. However, this detrimental effect caused by gamma benzene hexachloride was eliminated when experimental females were placed on normal food at the time of subsequent mating.

10. Ingestion of a diet containing 500 parts per million of gamma benzene hexachloride had no immediate effect on the lactating ability of normal nursing female rats when transferred to the

experimental diet.

11. The continual ingestion of a diet containing 500 parts per million of gamma benzene hexachloride did not produce any changes in either the erythrocyte or leucocyte counts in rats.

12. Experimental rats, after ingesting treated food for two months or more, showed an increase in the relative weight of the liver. An increase in kidney-body weight ratios was evident in all age groups of experimental males when compared to control males. Likewise, a significant increase in relative kidney weights was found in females maintained on the experimental diet for six months or more. Although there was a trend toward an increase in kidney weights of three month females that had been on the experimental diet for two months, the difference between the normal and experimental weights of this organ was not significant.

13. The relative increase in the liver-body weight ratio was similar in experimental males and females; however, the relative increase in kidney weight of experimental males (twenty-four to thirty-two per cent) was greater than the increase in experimental females (four to sixteen per cent). No significant changes were found in weights of spleen, adrenals, and testes in rats ingesting experimental food over prolonged periods.

14. Liver damage was found in rats ingesting experimental food. Rarely, however, was this damage great enough to be considered irreparable. Evidence of a chronic toxicity state in the liver was

indicated by albuminous and fatty degeneration. Generally, the kidneys appeared quite normal when examined histologically. In some kidney sections,nevertheless, some hyaline degeneration was evident.

15. Although maintained on a diet containing 500 parts per million of gamma benzene hexachloride for a year, rats were able to remove the toxicant from their tissues in a very short time when compared to the elimination of DDT from rat tissues. After twelve days on a normal diet, experimental females had only traces of gamma in their fat. On the twelfth day, the amounts of the toxicant in kidney, liver, blood, and muscle were apparently so low that the house fly bioassay test was not sensitive enough to detect any residual gamma.

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APPENDIX

Table I. History of normal litters.

Litter	Age of female (mos.)	Pair. to partur. (days)	Size of litter	Survivors at 3 days	Number weaned
1	3 1/2	22	6	4	4
2	4	22	3	3	3
3	4	24	9	4	4
4	4	24	9	9	9
5	4	26	4	4	4
6	5	21	11	10	10
7	5	21	8	8	0
8	5	23	9	8	6
9	5	24	8	6	6
10	5	24	8	8	8
11	5	24	12	6	0
12	5	24	9	9	9
13	6	23	10	9	9
14	6	26	6	6	6
15	6	24	11	11	11
16	6	25	5	5	5
17	6	24	11	5	0
18	6	24	4	3	3
19	6	27	7	7	7
20	7	22	9	9	9
21	7	23	7	7	7
22	7	24	6	6	6
23	7	24	9	9	9
24	7	25	8	8	8
25	7	25	11	11	6
26	7	26	10	10	10
27	7	27	11	11	11
28	7	37	11	11	11
29	8	23	11	11	11
30	8	25	11	11	11
31	9	24	6	0	0
32	9	25	7	7	7
33	9	33	7	7	7
34	10	21	8	8	6
35	10	25	11	11	7
36	11	28	8	0	0
37	12	37	4	2	0

Table II. History of experimental litters.

Litter	Age of female (mos.)	Length exp. diet (mos.)	Pair. to partur. (days)	Size of litter	Survivors at 3 days	Number weaned
G-1	3½	2	22	6	6	6
G-2	3½	3½	24	7	7	5
G-3	4	1	26	4	4	4
G-4	4	1½	21	8	6	6
G-5	4	3	22	7	0	0
G-6	4	4	24	5	2	0
G-7	4	4	24	5	0	0
G-8	4½	1½	23	5	0	0
G-9	4½	2	25	10	1	1
G-10	5	2	24	5	0	0
G-11	5	2	26	9	0	0
G-12	5	3	23	4	1	0
G-13	5	3	24	11	0	0
G-14	5	3	25	9	0	0
G-15	5	4	26	6	0	0
G-16	5	4	27	7	0	0
G-17	5	4	27	6	0	0
G-18	5	5	26	7	6	3
G-19	5	5	26	4	3	0
G-21	5½	2½	22	6	0	0
G-21	6	3	24	8	0	0
G-22	6	6	23	6	0	0
G-23	6	4	23	8	0	0
G-24	6	5	25	7	0	0
G-25	6	6	23	6	0	0
G-26	6	6	24	5	0	0
G-27	7	4	27	6	0	0
G-28	7	5	25	5	4	0
G-29	7	5	24	6	0	0
G-30	7	5	26	4	0	0
G-31	7	7	24	8	0	0
G-32	7	7	21	10	0	0
G-33	8	1	24	6	0	0
G-34	8	3½	28	8	0	0
G-35	8	7	24	8	0	0
G-36	8	8	27	9	0	0
G-37	8½	5	34	6	0	0
G-38	9	8	23	3	3	3
G-39	9	8	23	2	0	0
G-40	9	6	22	4	0	0
G-41	10	9	28	6	5	2
G-42	12	19	31	7	2	0
G-43	12	11	26	5	0	0
G-44	12	10	28	6	0	0

Table III. Organ-body weight ratios of normal male rats.

Rat	Age (mos.)	Body wt. (gms.)	Grams organ/kilogram body weight				
			Liver	Kidneys	Spleen	Adrenals	Testes
101	3½	230	45.3	8.8	2.64	0.11	11.7
102	3½	245	44.5	8.8	1.13	0.11	12.7
103	3½	227	41.4	8.9	2.75	0.11	-*
201	7	418	31.0	8.5	1.70	0.14	9.1
202	7	295	29.0	8.2	2.00	0.12	11.1
203	7	315	29.8	8.7	1.72	0.10	14.4
204	7	318	31.5	8.5	1.35	0.09	11.4
205	7	400	40.6	11.1	1.85	0.14	14.9
206	7	357	33.4	7.3	1.28	0.08	9.2
207	7	280	34.8	7.7	1.39	0.09	11.2
208	7	308	31.6	7.7	1.40	0.08	9.2
209	7	360	29.9	8.7	2.01	0.09	11.3
210	7	355	39.0	8.4	2.45	0.08	8.4
211	7	294	29.3	6.9	1.56	0.09	9.4
212	7	356	37.5	8.3	1.88	0.11	10.2

*Only one testis.

Table IV. Organ-body weight ratios of experimental male rats.

Rat	Age (mos.)	Exp. diet (mos.)	Body wt. (gms.)	Grams organ/kilogram body weight				
				Liver	Kidneys	Spleen	Adrenals	Testes
151	3½	2	251	50.2	11.3	1.70	0.09	12.0
152	3½	2	249	49.6	11.7	2.82	0.14	11.8
153	3½	2	205	53.0	11.7	1.78	0.13	12.5
251	7	5	290	36.0	10.2	2.20	0.14	11.5
252	7	5	368	43.0	9.6	1.93	0.08	9.1
253	7	6	355	44.1	10.8	1.94	0.11	9.6
254	7	6	362	42.0	9.4	2.00	0.09	6.0
255	7	6	366	34.5	10.3	2.40	0.12	9.3
256	7	6	315	35.3	9.9	2.16	0.09	7.4
257	7	6	314	41.8	10.6	1.50	0.08	9.0
258	7	6	355	35.0	8.8	2.28	0.10	9.9
259	7	6	290	42.7	12.2	1.42	0.09	10.4
260	7	6	302	45.7	10.0	1.85	0.09	14.2
261	7	6	408	46.5	11.3	1.76	0.11	9.6

Table V. Organ-body weight ratios of normal female rats.

Rat	Age (mos.)	Body wt. (gms.)	Grams organ/kilogram body weight			
			Liver	Kidneys	Spleen	Adrenals
151	3	178	41.3	8.8	2.8	0.28
152	3	184	43.5	8.7	3.0	0.27
153	3	201	45.5	8.5	2.6	0.26
154	3	187	40.4	8.2	2.4	0.27
155	3	200	38.7	9.1	2.5	0.28
156	3	180	38.2	10.0	2.8	0.28
221	7	200	32.6	7.4	1.8	0.15
222	7	230	35.1	7.6	1.5	0.14
223	7	230	36.6	7.9	1.5	0.16
224	7	196	38.1	8.3	1.8	0.16
225	7	185	36.3	8.7	1.9	0.18
226	7	252	34.1	8.3	2.4	0.21
227	7	137	33.4	8.3	2.3	0.25
321	18	220	38.1	10.6	1.9	0.15
322	18	270	32.2	10.5	2.1	0.15
323	18	210	34.4	8.3	2.7	0.18
324	18	252	34.1	8.3	2.4	0.21

Table VI. Organ-body weight ratios of experimental female rats.

Rat	Age (mos.)	Exp. diet (mos.)	Body wt. (gms.)	Grams organ/kilogram body weight			
				Liver	Kidneys	Spleen	Adrenals
171	3	2	173	54.0	8.8	3.3	0.24
172	3	2	174	48.7	9.6	3.2	0.28
173	3	2	220	52.4	8.5	2.4	0.21
174	3	2	196	46.0	9.1	2.6	0.22
175	3	2	220	49.7	9.3	2.0	0.25
176	3	2	185	51.2	10.2	2.5	0.30
281	7	3*	208	38.5	7.9	2.2	0.17
282	7	3*	200	38.9	8.8	2.8	0.23
283	7	3*	220	38.1	8.6	2.5	0.23
284	7	3*	244	41.6	9.4	2.1	0.21
271	7	6	226	42.1	8.8	2.1	0.23
272	7	6	180	45.8	8.8	2.3	0.23
273	7	6	182	44.1	9.1	1.7	0.22
274	7	6	198	40.0	8.8	2.0	0.20
371	18	17	180	44.8	11.9	2.0	0.16
372	18	17	256	51.1	10.4	1.8	0.18
373	18	17	165	56.1	12.6	2.3	0.23
374	18	17	230	41.1	11.2	1.8	0.18
375	18	17	204	39.3	10.0	2.0	0.18
376	18	17	212	41.9	11.6	1.6	0.25
377	18	17	219	40.5	10.0	1.7	0.17

* After weaning, rat on experimental ration 3 months, then on normal ration 3 months prior to autopsy.